METHOD AND SYSTEM FOR INTRODUCING
A GENE INTO A HUMAN STEM CELL
INV.: K. WANG
DKT.: 258/193

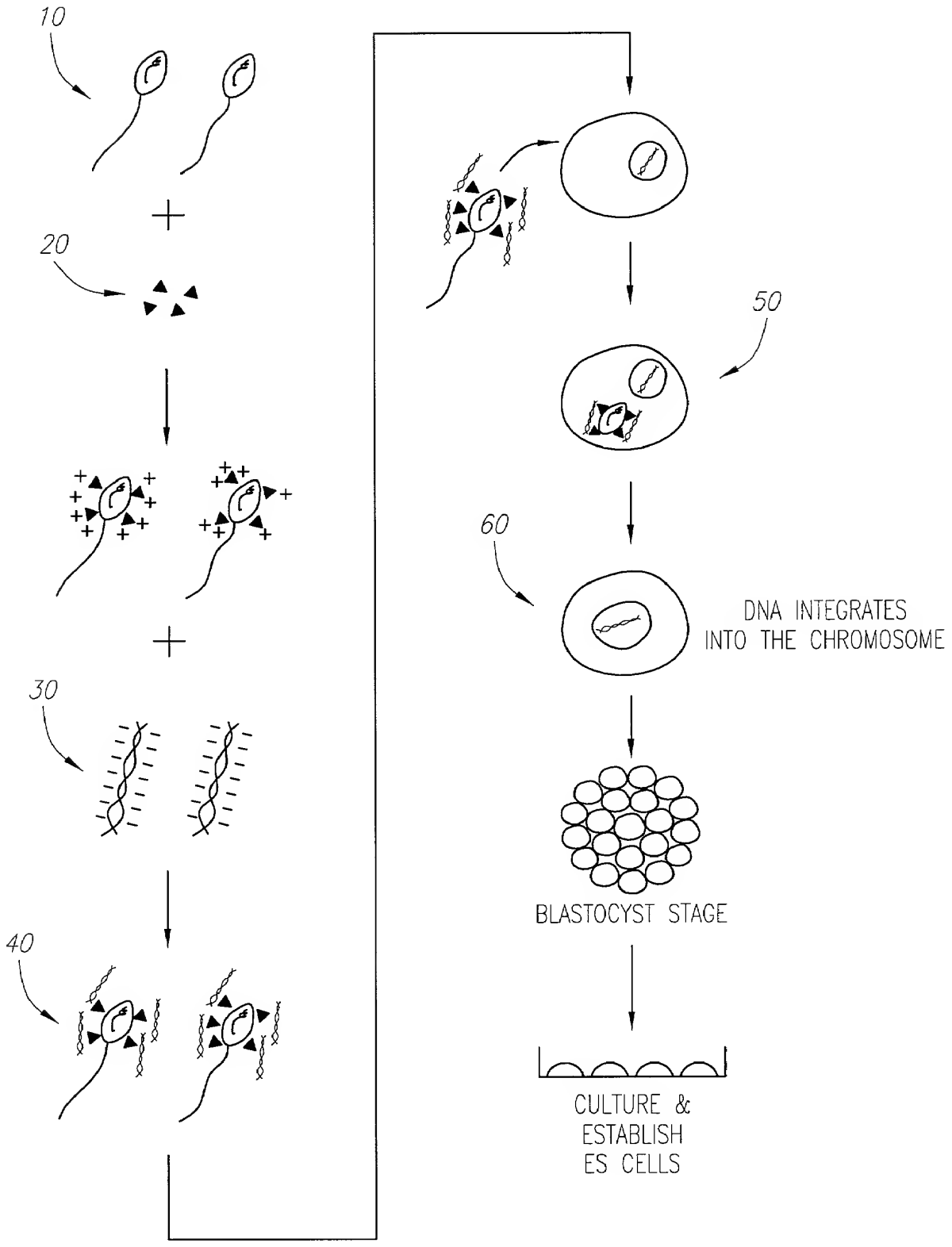


Fig. 1

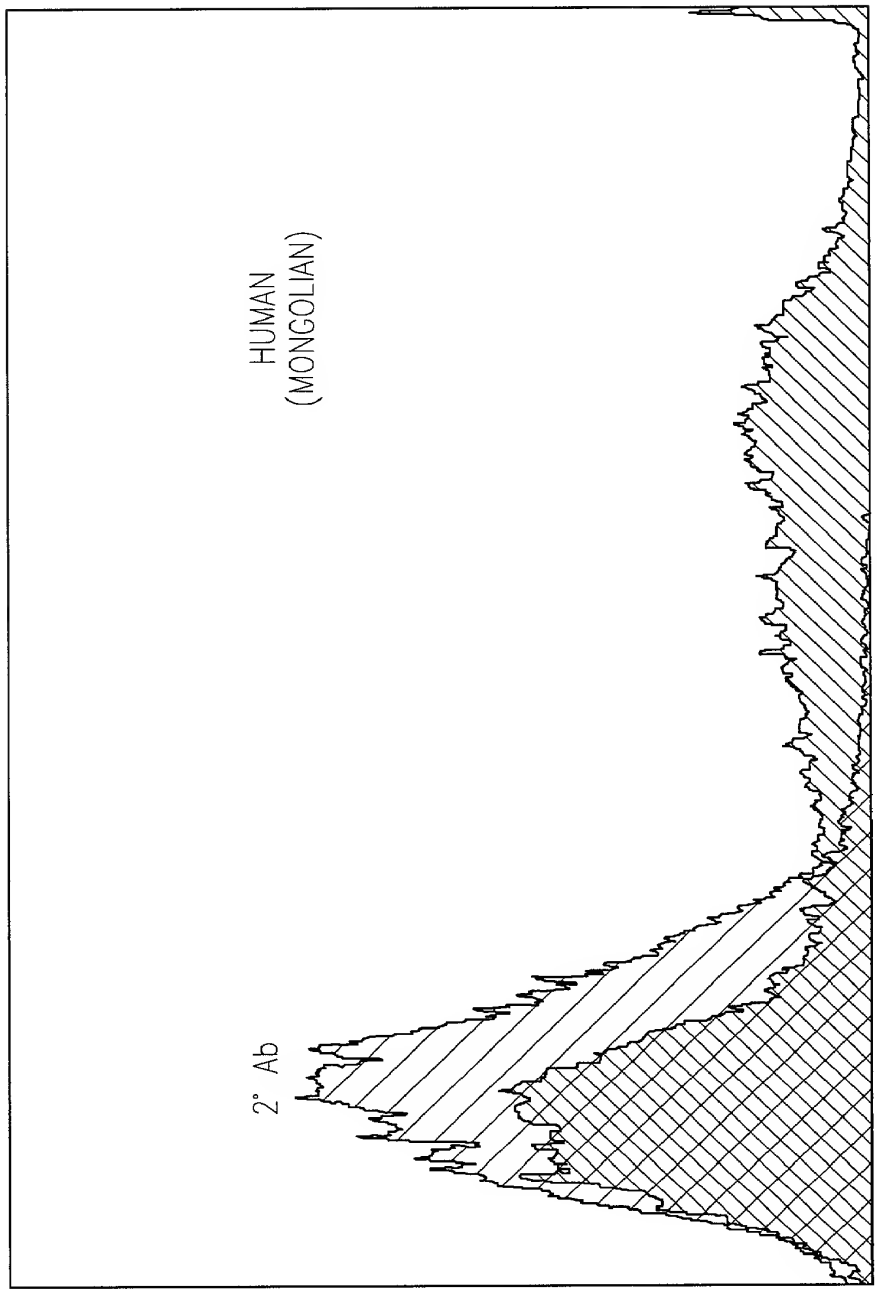

FLOW CYTOMETRY ANALYSES FOR mAb C BINDING
TO HUMAN SPERM CELL
HUMAN
(MONGOLIAN)
2° Ab

Fig. 2

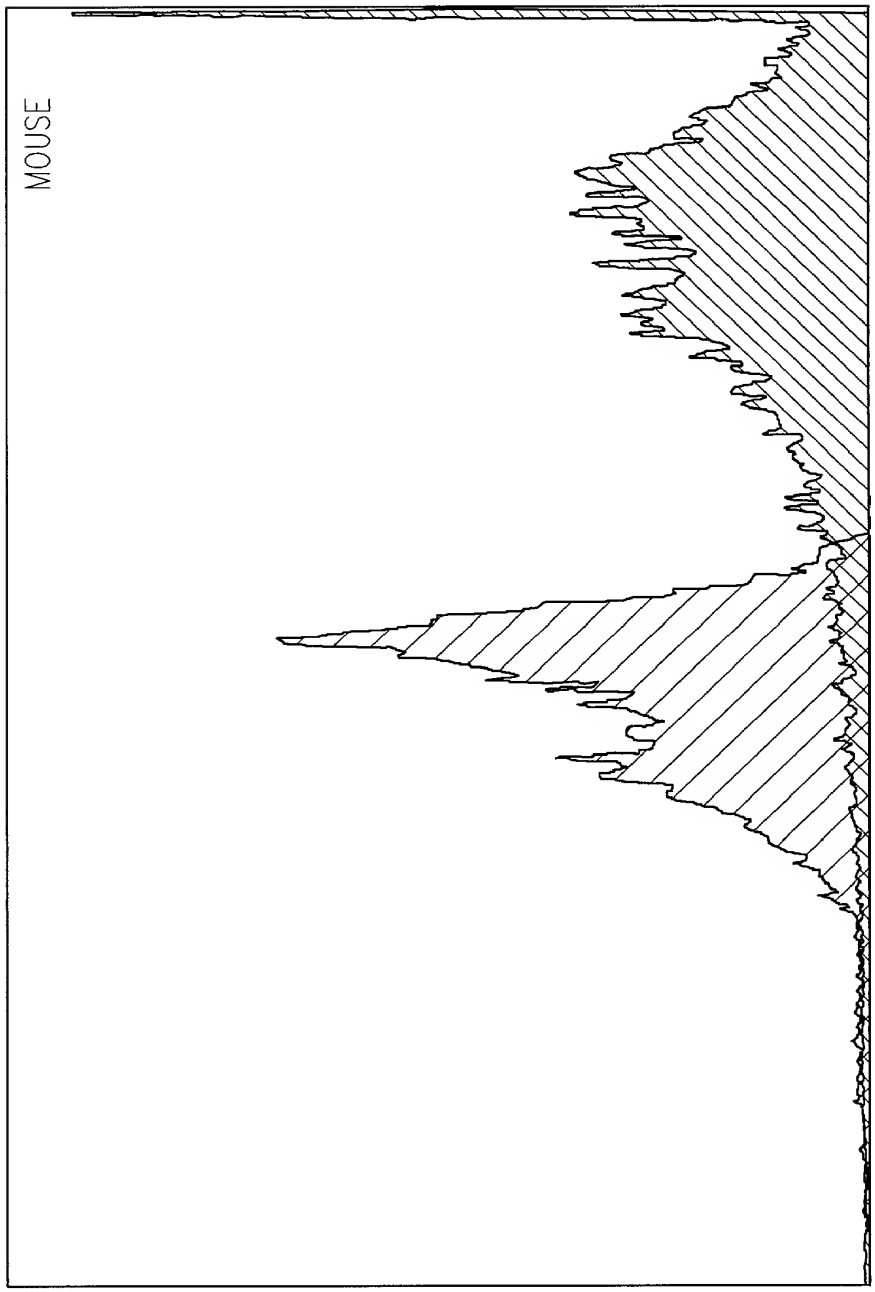


Fig. 3

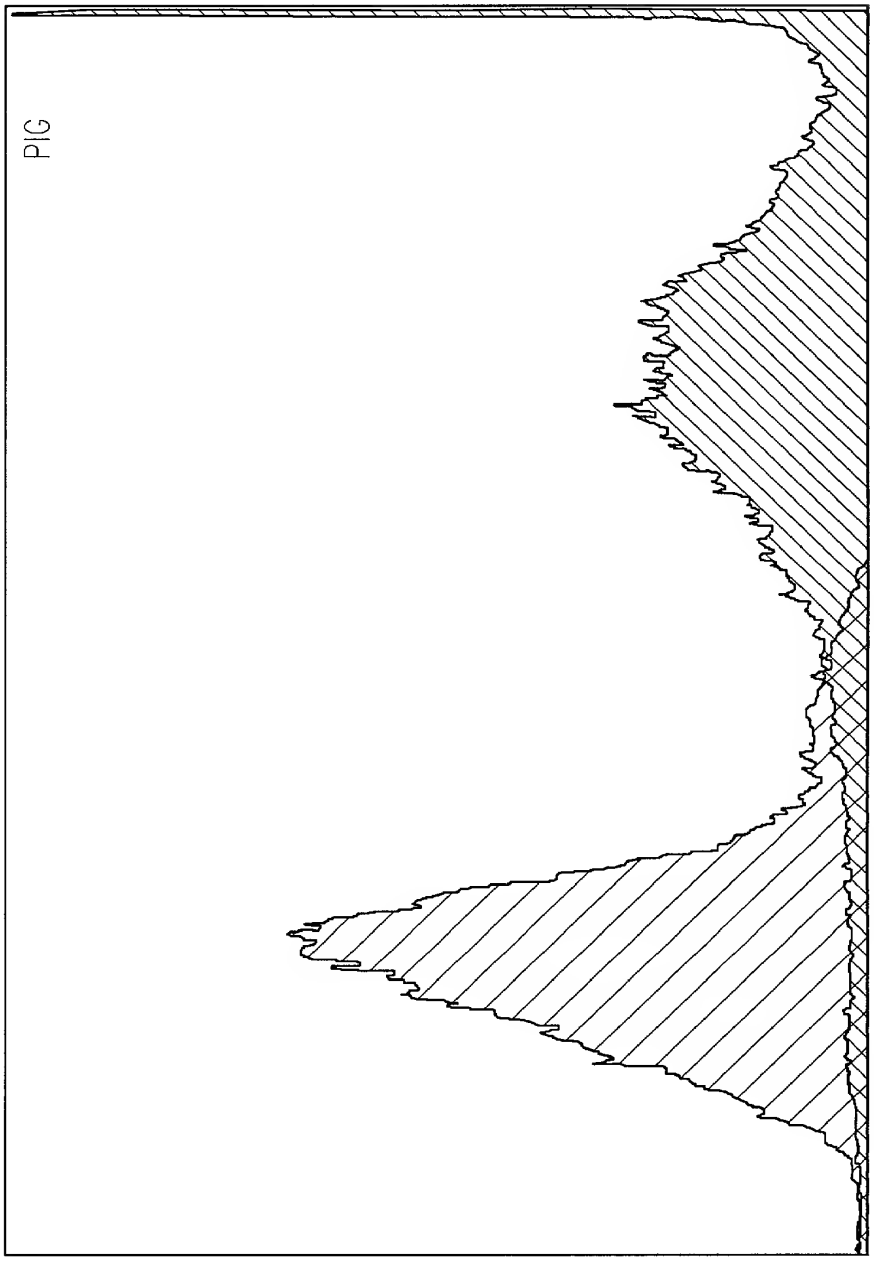
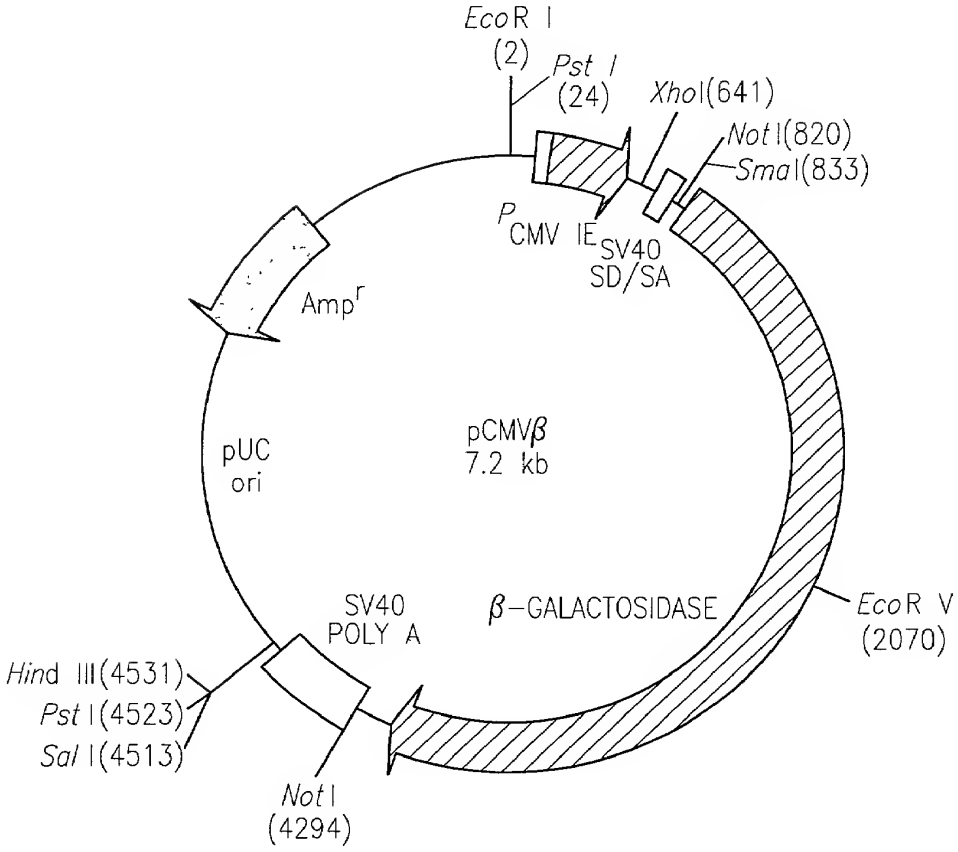


Fig. 4



RESTRICTION MAP OF pCMVβ. UNIQUE RESTRICTION SITES ARE BOLD.

DESCRIPTION:

pCMVβ IS A MAMMALIAN REPORTER VECTOR DESIGNED TO EXPRESSION β–GALACTOSIDASE IN MAMMALIAN CELLS FROM THE HUMAN CYTOMEGALOVIRUS IMMEDIATE EARLY GENE PROMOTER (1). pCMVβ CONTAINS AN INTRON (SPLICE DONOR/SPLICE ACCEPTOR;2) AND POLYADENYLATION SIGNAL FROM SV40, AND THE FULL LENGTH *E. COLI* β–GALACTOSIDASE GENE WITH EUKARYOTIC TRANSLATION INITIATION SIGNALS (3). pCMVβ EXPRESSES HIGH LEVELS OF β–GALACTOSIDASE AND CAN BE USED AS A REFERENCE (CONTROL) PLASMID WHEN TRANSFECTING OTHER REPORTER GENE CONSTRUCTS AND CAN BE USED TO OPTIMIZE TRANSFECTION PROTOCOLS BY EMPLOYING STANDARD ASSAYS OR STAINS TO ASSAY β–GALACTOSIDASE ACTIVITY. ALTERNATIVELY, THE β–GALACTOSIDASE GENE CAN BE EXCISED USING THE *Not* I SITES AT EACH END TO ALLOW OTHER GENES TO BE INSERTED INTO THE pCMVβ VECTOR BACKBONE FOR EXPRESSION IN MAMMALIAN CELLS OR TO INSERT THE β–GALACTOSIDASE FRAGMENT INTO ANOTHER EXPRESSION VECTOR.

Fig. 5

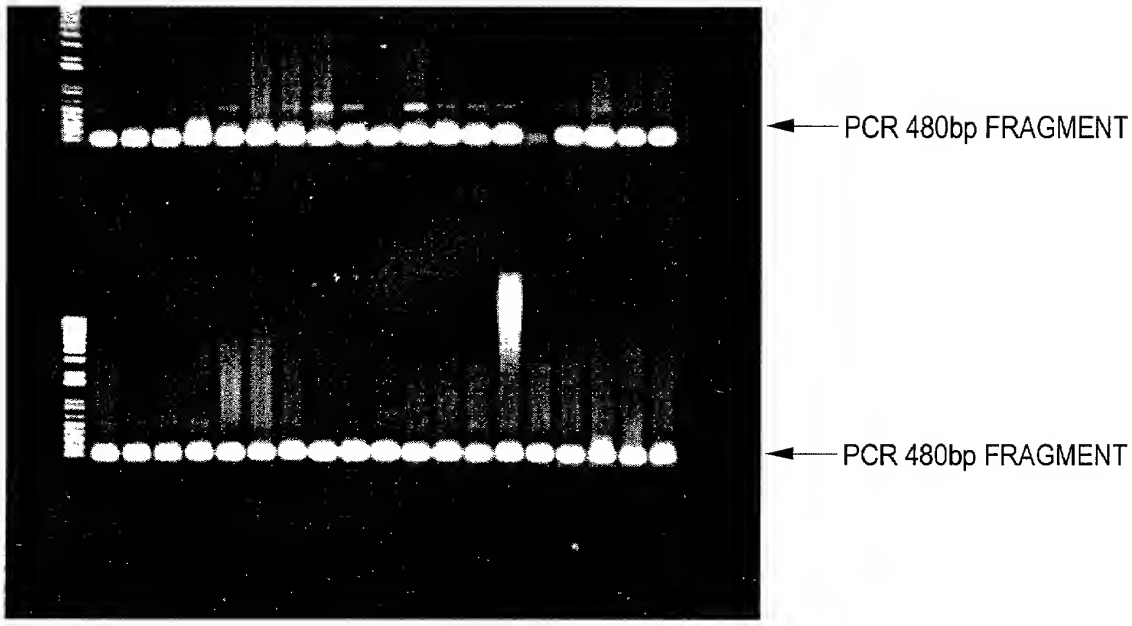


Fig. 6

HBsAG SOUTHERN BLOT
3 DAY EXPOSURE

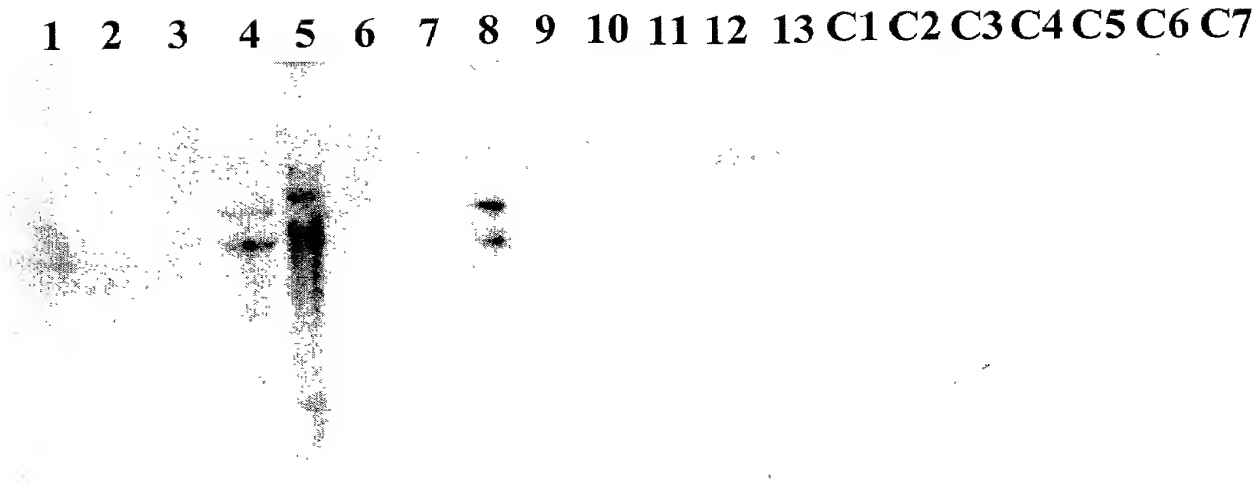
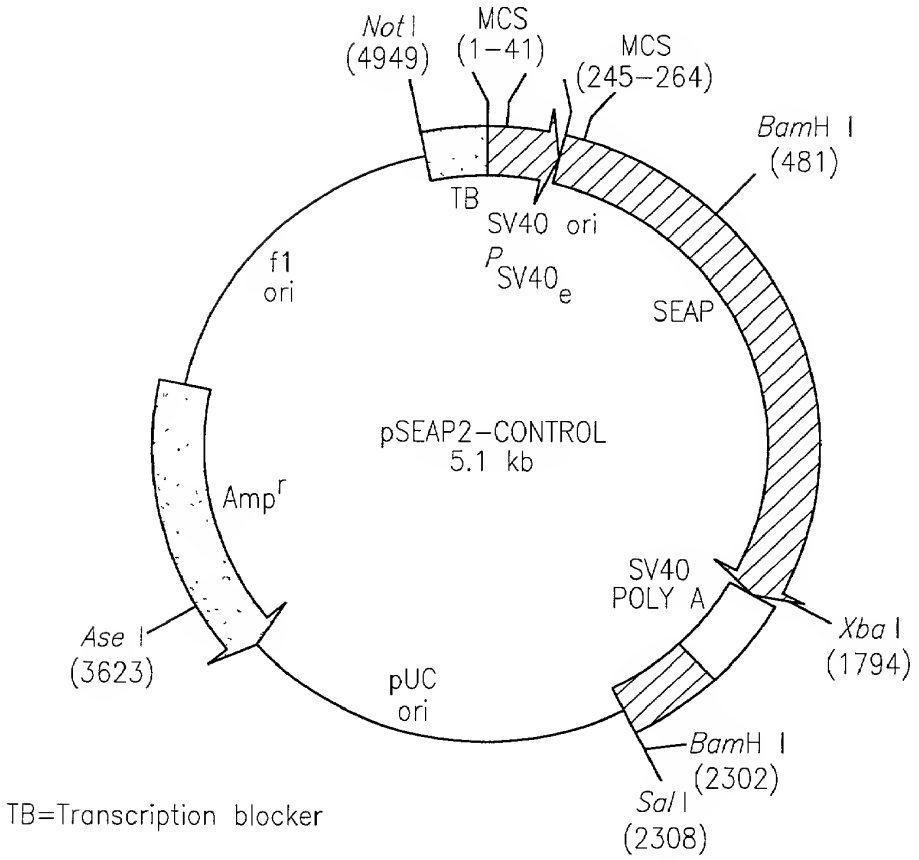


Fig. 7



RESTRICTION MAP AND MULTIPLE CLONING SITE (MCS) OF pSEAP2–CONTROL UNIQUE RESTRICTION SITES ARE BOLD.

DESCRIPTION:

pSEAP2–CONTROL IS A POSITIVE CONTROL VECTOR EXPRESSING SECRETED ALKALINE PHOSPHATE (SEAP) UNDER THE CONTROL OF THE SV40 EARLY PROMOTER AND THE SV40 ENHANCER. THE SEAP CODING SEQUENCE IS FOLLOWED BY THE SV40 LATE POLYADELYNATION SIGNAL TO ENSURE PROPER, EFFECIENT PROCESSING OF THE SEAP TRANSCRIPT IN EUKARYOTIC CELLS. A SYNTHETIC TRANSCRIPTION BLOCKER (TB), COMPOSED OF ADJACENT POLYADENYLATION AND TRANSCRIPTION PAUSE SITES, LOCATED UPSTREAM OF THE MCS REDUCES BACKGROUND TRANSCRIPTION (1). THE VECTOR BACKBONE ALSO CONTAINS AN F1 ORIGIN FOR SINGLE–STRANDED DNA PRODUCTION, A pUC ORIGIN OF REPLICATION, AND AN AMPICILLIN RESISTANCE GENE FOR PROPAGATION AND SELECTION IN *E. COLI*. THE SEAP2 VECTORS INCORPORATE A NUMBER OF FEATURES THAT IMPROVE THE SENSITIVITY OF SEAP BY INCREASING THE EFFICIENCY OF SEAP EXPRESSION OR THAT ENHANCE THE UTILITY OF THE VECTORS. THESE ICLUDE: AN IMPROVED KOZAK CONSENSUS TRANSLATION INITIATION SITE (2); THE REMOVAL OF THE SV40 SMALL–T INTRON, WHICH CAN CAUSE CRYPTIC SPLICING AND REDUCED EXPRESSION IN SOME GENES AND/OR CELL TYPES (3,4); SWITCHING FROM THE EARLY TO LATE POLYADENYLATION SIGNAL OF SV40, WHICH TYPICALLY CAUSES A FIVE–FOLD INCREASE IN mRNA LEVELS (5); AN EXPANDED MULTIPLE CLONING SITE (MCS); COMPACT PLASMID SIZE; AND REMOVAL OF EXTRANEOUS SEQUENCES FROM THE 3' UNTRANSLATED REGION OF THE SEAP mRNA

Fig. 8

Pig Tail DNA Southern Blot

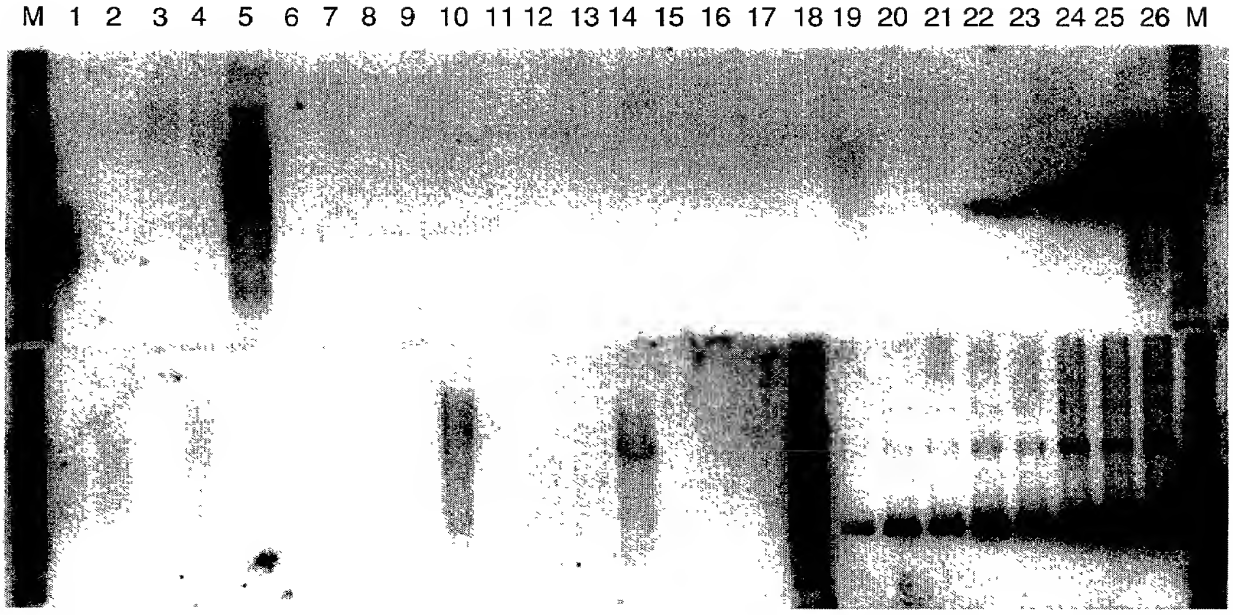


Fig. 9

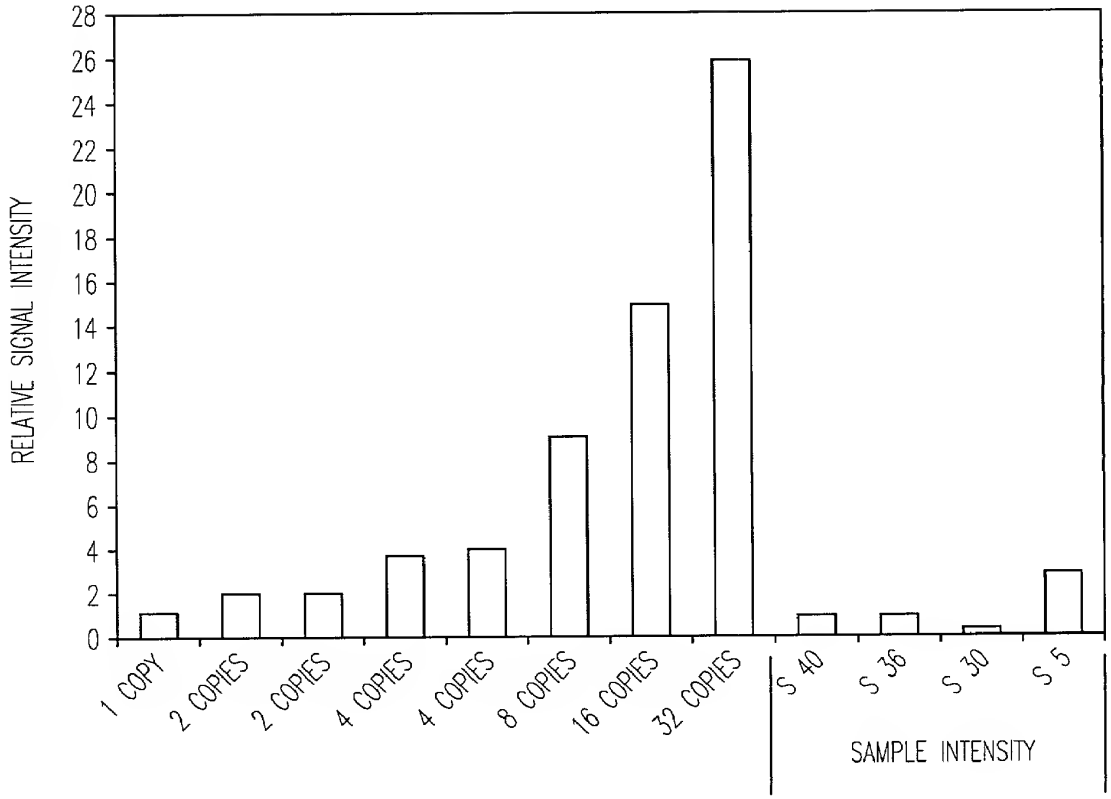

RELATIVE SIGNAL INTENSITY
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 COPY
2 COPIES
2 COPIES
4 COPIES
4 COPIES
8 COPIES
16 COPIES
32 COPIES
S 40
S 36
S 30
S 5
SAMPLE INTENSITY

Fig. 10

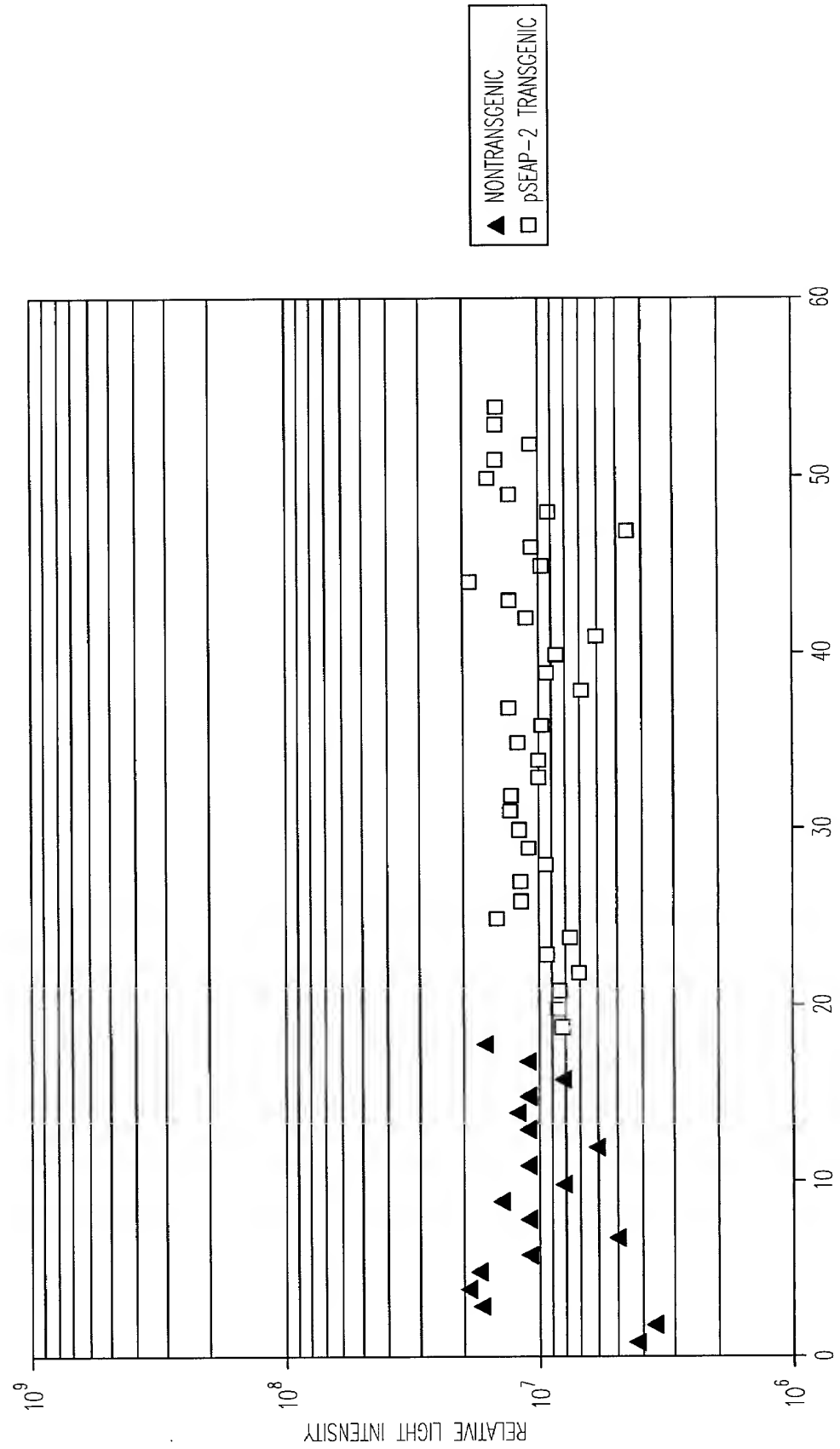

NONTRANSGENIC
pSEAP-2 TRANSGENIC
RELATIVE LIGHT INTENSITY
10^9
10^8
10^7
10^6
0
10
20
30
40
50
60
PIG NUMBER

Fig. 11

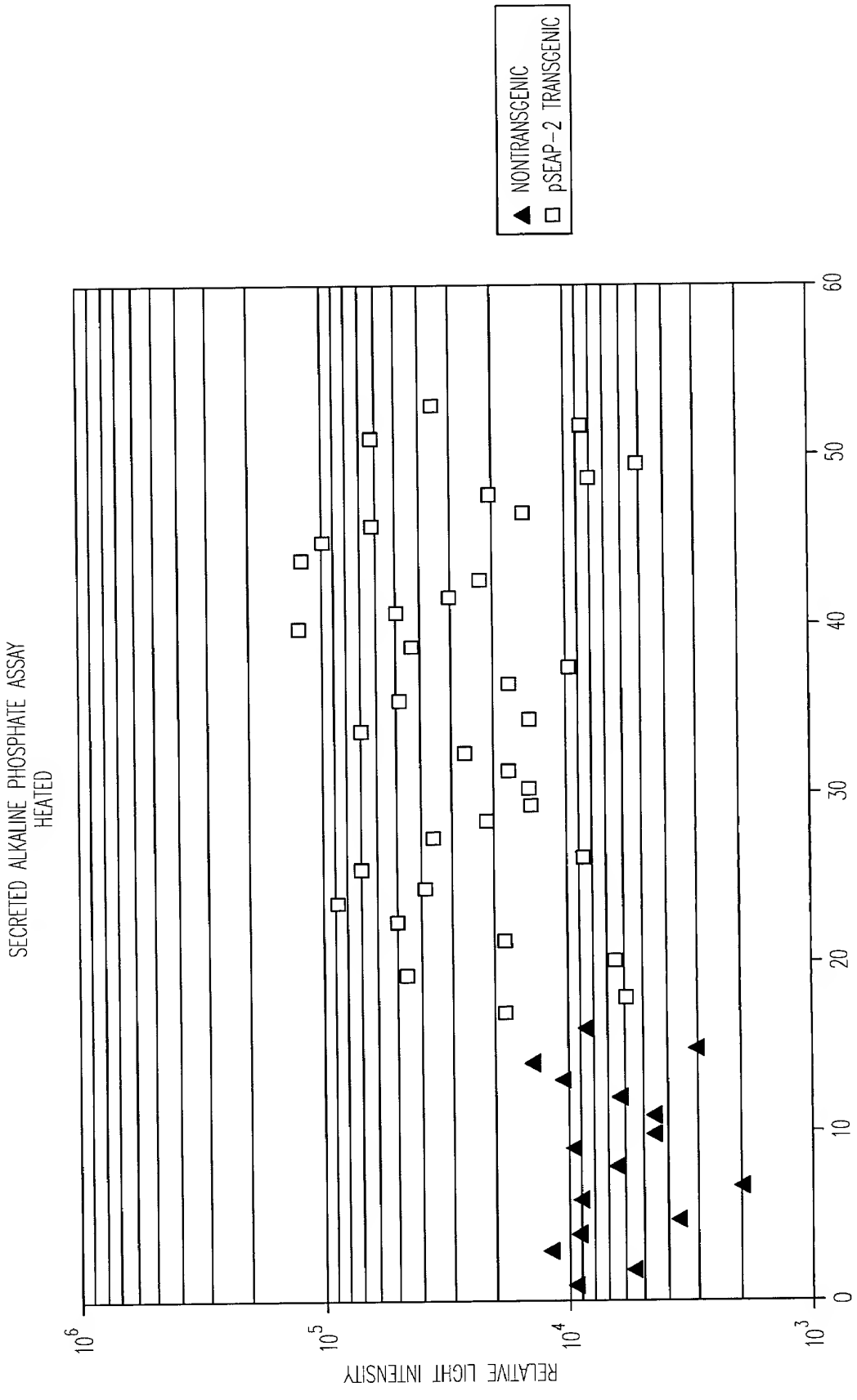

NONTRANSGENIC
pSEAP-2 TRANSGENIC
SECRETED ALKALINE PHOSPHATE ASSAY
HEATED
RELATIVE LIGHT INTENSITY
10^6
10^5
10^4
10^3
0
10
20
30
40
50
60
PIG NUMBER

Fig. 12